Mapping the Elements of Cellular Life and Disease

Metal Ion Relocation in Pathways in Cell Signaling and Morphogenesis

Lydia Finney, Suneeta Mandava, Lyann Ursos, Wen Zhang, Diane Rodi, Stefan Vogt,* Daniel Legnini,* and David Glesne
Biosciences Division and *Experimental Facilities Division

Abstract

To sustain life, a delicate balance between the essential and toxic character of endogenous metal ions must be achieved. Although much has been learned about this homeostasis, our knowledge of how metal localization changes in eukaryotic cells during various life processes is very limited.

For example, angiogenesis, the cellular branching process by which new blood capillaries are formed, has long been known to be sensitive to copper, but without any clear explanation. We here utilize recent developments in x-ray optics to investigate the regulation of copper content both *in vitro* and *in vivo* and find a surprisingly dramatic spatial relocalization of 80-90% of cellular copper stores from intracellular compartments to growing endothelial cell filopodia during angiogenesis. Preliminary studies have also revealed dynamics in the localization of metal ions in other systems.

Motivation

While many of the roles of metalloproteins, such as in catalysis and oxygen transport are well known, much more of their biology remains unknown. Where are metal ions localized within cells? Does this change as cells go through different processes in their life cycle or in response to cell signals as in angiogenesis? The technical challenges of visualizing metal ions in cells has hindered our ability to solve many problems related to disease,

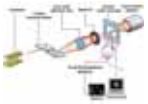
But recent developments in x-ray optics at third-generation synchrotron sources and the development of highly sensitive and selective fluorescent indicators and chelators of metal ions have provided resources for highly sensitive visualization and quantitation of metalloproteins in biological samples. Many long-standing questions in the biology and medicine of metal ions may now be addressed.

Methods

*Detailed optimization of conditions for tubulogenesis of primary human microvascular endothelial cells has been developed

*For x-ray microscopy, cells are adhered in the presence or absence of substrate to silicon nitride windows or carbon-formvar on gold grids, stained, imaged optically, fixed, and dried

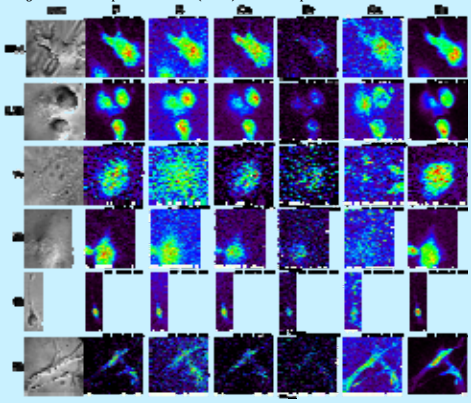
.



Metal Ion Localization during Angiogenesis: a Model Study in HMVEC Cells

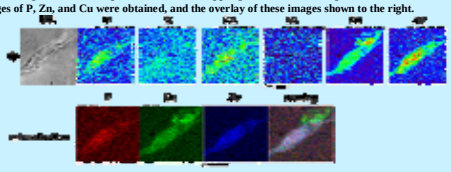
XFM scans of HMVEC cells undergoing tubulogenesis

Silicon nitride windows were coated with either a thin layer of Matrigel™ or a layer of gelatin. HMVEC cells were plated on these substrates and exposed to VEGF and bFGF. The process was stopped at various times subsequent to initiation, the cells fixed, and both light microscope and XFM images obtained of representative cells (n=>16) at each time point.

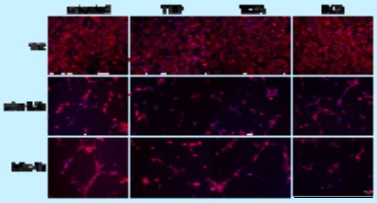


Role of copper in HMVEC cell filopodia extension

Areas at the tips of HMVEC filopodia extensions were scanned by XFM at high resolution. The optical image and metal maps are shown in the upper panel. In the lower panel, false color images of P, Zn, and Cu were obtained, and the overlay of these images shown to the right.



Chelation of copper disrupts tubulogenesis of HMVEC cells



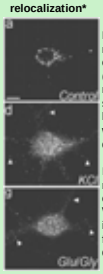
HMVEC cells were plated in either gelatin or Matrigel™-coated dishes, stimulated with bFGF and VEGF, and untreated or exposed to 100 µM TEPA, 100 µM TTM, or 100 µM BCS. The cells were incubated for either 1.5 or 4 h, fixed and permabilized, and stained with Hoescht 33342 and Alexa Fluor 660-phalloidin. Cells were then imaged and Hoescht signal pseudocolored blue and phalloidin pseudocolored red.

Visualizing Roles of Metals in Neuronal Growth and Receptor Stimulation

XFM Scans of Primary Hippocampal Neurons during NMDA Receptor Stimulation

Silicon nitride windows were coated with poly-D-lysine and laminin. Primary hippocampal neuronal cells were plated on this substrate and, in stimulated case, exposed to glutamate/glycine. The process was stopped, the cells fixed, and both light microscope and XFM images obtained of representative cells.

MNK relocalization*

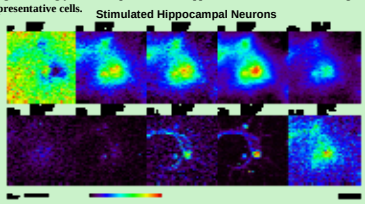


Recently the relocalization of a copper transporter (MNK) to plasma membrane upon stimulation of hippocampal neurons with glutamate was demonstrated

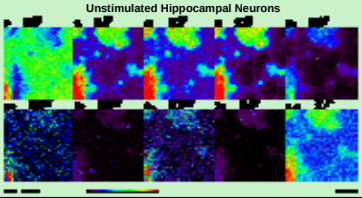
Is Copper relocalization concommitant with WND relocalization? Is it a more general feature of neuronal depolarization?

* Schlief ML, Craig AM, Gitlin, JD. NMDA receptor activation mediates copper homeostasis in hippocampal neurons. (2005) J. Neurosci. 25(1): 239-246.

Stimulated Hippocampal Neurons

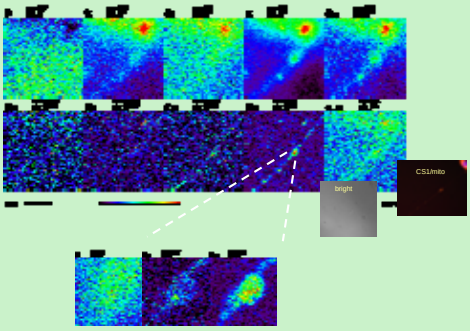


Unstimulated Hippocampal Neurons



Comparative analysis with neurite outgrowth

B103 neuroblastoma cells (Schubert, 1974) were grown on silicon nitride windows. Neurite outgrowth was induced with 1 mM dbCAMP and 1% FCS. The cells were fixed, and both light microscope and XFM images obtained of representative cells. The localization of metal ions appears different from that of either that of stimulated neurons or endothelial cells that are putting out processes.



Results and Discussion

These studies suggest a dynamic model of cellular transition metal localization. In endothelial cells, we see that at least some of the copper that plays a role in regulating tubulogenesis is arising from intracellular stores. High resolution scans of areas of punctate copper signal at the periphery of growing filopodia indicate that the copper may be secreted from the cells. Additionally, prelimainary studies in a neuronal system suggest that relocalization of metal ions may occur during stimulation.

While such a dynamic model has been well established for calcium, a similar role for transition metals such as copper and zinc has far less experimental support. This may be a result of an historical inability to sensitively measure and spatially localize these metals unlike calcium for which sensitive, selective, and optically visual probes have been available for 25 years.

Next Steps & New Questions

These studies lead us in new directions:

- What proteins are involved in the secretion of copper during tubulogenesis? What clues does this give us to the role of copper in the process of angiogenesis?
- Can we advance XRF microscopy techniques through the development of subcellular nonendogenous-metal XRF indicators?
- What is the role of metal ion relocalization in the cell? Are other cell life processes involved in the dynamic regulation of metal ion partitioning in the cell?

For more info…

Check out our webpage:
http://www.bio.anl.gov/functionalgenomics/metalloproteomics.html

Or the Experimental Facilities Division Page:
http://www.aps.anl.gov/Experimental_Facilities_Division/index.html

Or just ask Lydia:

Research funded by U.S. Department of Energy

A U.S. Department of Energy laboratory managed by The University of Chicago